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Adenovirus-mediated Wild-Type *p53* Gene Transfer as a Surgical Adjuvant in Advanced Head and Neck Cancers[1]

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ABSTRACT

A high incidence of locoregional failure contributes to the poor overall survival rate of around 50% for patients with squamous cell carcinoma of the head and neck (SCCHN). *In vitro* and *in vivo* preclinical work with adenovirus-mediated wild-type *p53* gene transfer using the recombinant *p53* adenovirus (Ad-*p53*) has shown its promise as a novel intervention strategy for SCCHN. These data have translated into Phase I and Phase II studies of Ad-*p53* gene transfer in patients with advanced, locoregionally recurrent SCCHN. The safety and overall patient tolerance of Ad-*p53* has been demonstrated. Of 15 resectable but historically noncurable patients in the surgical arm of a Phase I study, 4 patients (27%) remain free of disease, with a median follow-up time of 18.25 months. Surgical and gene transfer-related morbidities were minimal. These results provide preliminary support for the use of Ad-*p53* gene transfer as a surgical adjuvant in patients with advanced SCCHN. The implications of our findings for the management of SCCHN in general are discussed.

INTRODUCTION

The treatment of advanced primary human SCCHN[3] in the upper aerodigestive tract remains a major therapeutic challenge, despite advances in surgical and radiotherapeutic techniques. Locoregionally recurrent disease, which has a particularly dismal prognosis and few meaningful treatment options, remains the principal cause of death among patients with advanced SCCHN (1, 2). In addition, it has been shown that detection of clonal specific *p53* mutations at tumor margins in SCCHN is a predictor of local recurrence (3, 4). These molecular pathological advances suggest that despite adjuvant radiotherapy, residual disease (microscopic as well as histologically normal but genotypically abnormal) is a major problem in the treatment of patients with SCCHN. Our interest in developing new treatment strategies for SCCHN is generated by the humbling overall survival rate of approximately 50%, which has not changed over the last several decades (5).

Mutation of the *p53* tumor suppressor gene is one of the most common genetic alterations in human malignancy (6). Approximately 60% of human tumors are thought to possess mutation at the *p53* locus. Transient overexpression of the wild-type *p53* gene in various malignancies has been considered a potential molecular intervention strategy (7–12). This strategy is based on the role that wild-type *p53* plays as a tumor suppressor gene and an inducer of cell cycle arrest and apoptosis (6, 13–16). Our laboratory has focused on the potential of wild-type *p53* gene transfer as a strategy for the selective induction of apoptosis in SCCHN. The recombinant adenovirus Ad-*p53* has been used as the gene delivery tool in all of our preclinical studies (7–9). The tropism of the adenovirus for tissues of the upper aerodigestive tract, the ability to produce the adenovirus in high titers, and the efficiency of adenovirus-mediated gene transfer have made this vector an attractive tool for transient gene delivery.

In our preclinical studies with Ad-*p53*, transduction of wild-type *p53* into several different SCCHN cell lines induced apoptosis without adversely affecting normal cells (7, 8). We have also shown that Ad-*p53* reduces the growth of established tumors in xenograft models of SCCHN (8). Additionally, we have demonstrated that in a nude mouse xenograft model of microscopic residual disease, Ad-*p53* can prevent the establishment of tumors from subcutaneously deposited SCCHN cell lines in a dose-dependent fashion (7).

In our recently completed Phase I clinical trial of Ad-*p53* gene transfer in patients with advanced locoregionally recurrent SCCHN who were unsuccessfully treated with conventional therapy including radiotherapy, two treatment arms were established. Our previous report (17) demonstrated the feasibility and tolerance of Ad-*p53* administered to patients with nonresectable disease and to patients who could be surgically treated but were historically deemed incurable; tissue vector biodistribution was evaluated in this publication as well. In this current focused analysis with longer patient follow-up (median follow-up, 18.25 months), we report the potential antitumor activity and complications of Ad-*p53* in a surgical adjuvant setting (the surgical treatment arm), based on our Phase I experience.

MATERIALS AND METHODS

Study Subjects. Of the 33 total patients entered into the Phase I study, 15 patients with advanced locoregionally recur-

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[3] The abbreviations used are: SCCHN, squamous cell carcinoma of the head and neck; pfu, particle-forming unit; TdT, terminal deoxynucleotidyltransferase.

Table 1 Demographics of surgical treatment arm study participants

Patient no.	Age (yr)	Sex	*p53* genotype	Index tumor site	Prior treatments related to index tumor	Recurrent tumor site
1	31	F	Mutant	Tongue, floor of mouth	1. laser exc. L. tongue[a] 2. XRT to oral cavity 3. L. hemiglossectomy, mandibulectomy, MRND 4. 1 cycle CDDP & 5FU	Left face and neck
2	58	M	Mutant	Supraglottic larynx	1. supraglottic laryngectomy 2. completion laryngectomy, bilateral MRND 3. XRT to bilateral necks	Submental and submaxillary area
3	72	M	WT	Unknown primary	1. L. MRND 2. XRT to L. neck and supraclavicular region (5400 cGY)	Left neck
4	46	M	Mutant	Tongue base	1. L. RND and R. cervical node biopsy 2. XRT to L. and R. necks and tongue base (6600 cGy)	Tongue base
5	58	M	Mutant	Larynx	1. wide field laryngectomy 2. XRT to larynx (6300 cGy)	Hypopharynx
6	48	M	Mutant	Tongue base	1. XRT to tongue base, chemotherapy	Right superior larynx
7	76	F	Mutant	Supraglottic larynx	1. XRT to larynx, retropharyngeal and subdigastric nodes	Left supraclavicular area
8	53	M	WT	Unknown primary	1. R. RND 2. XRT to R. neck (5400 cGy) 3. XRT to submentum (4600 cGy) 4. 2 cycles cisplatin	Right submentum
9	56	M	Mutant	Larynx	1. verticle hemilaryngectomy 2. XRT to anterior neck and larynx (5500 cGy) 3. total laryngectomy	Neopharynx and peristomal region
10	49	F	NE	Left oral tongue	1. wide local exc. L. tongue 2. XRT to L. supraclavicular area (5040 cGy), L. neck (5600 cGy), and tongue and floor of mouth (3600 cGy)	Tongue
11	68	M	Mutant	Left tonsil	1. XRT to L. tonsil, L. upper neck, L. supraclavicular fossa (7000 cGy)	Left tonsil
12	37	M	Mutant	Left oral tongue	1. hemiglossectomy 2. re-excision L. tongue 3. XRT to tongue (6400 cGy) 4. L. MND	Left tongue base
13	34	F	NE	Floor of mouth and submentum	1. wide local exc. tongue and floor of mouth, L. MRND 2. 2 cycles of Taxol, ifosphamide, cisplatin 3. XRT and 2 cycles of 5FU and cisplatin	Left tongue and floor of mouth
14	56	M	Mutant	Right hypopharynx	1. 2 cycles of 5FU and cisplatin 2. partial laryngopharyngectomy, R. RND 3. XRT to R. neck (6300 cGy)	Right hypopharynx
15	73	F	Mutant	Left buccal mucosa	1. exc. L. buccal lesion 2. exc. L. retromolar trigone 3. L. hemimandibulectomy, L. MRND 4. XRT to L. cheek, face, and neck (4500 cGy) 5. XRT boost (900 cGy) to L. cheek	Left buccal mucosa

[a] L., left; R., right; exc., excision; XRT, radiation therapy; CDDP, cis-diamminedichloroplatinum; 5FU, 5-fluorouracil; MRND, modified radical neck dissection; RND, radical neck dissection; WT, wild-type; NE, could not be evaluated. Recurrent tumor site refers to the recurrent lesion that was treated in this Phase I trial.

rent or refractory SCCHN were placed into the surgical treatment arm. These 15 patients are the subjects of this report. For this report, we also examined biopsy samples of tumor margin and untreated adjacent normal tissues from a representative nonsurgical patient for evidence of apoptosis as well as expression of the wild-type p53 and p21^{Waf1} gene products.

Patients typically had multiple treatments for either refractory or locoregionally recurrent disease before study entry (Ta-

ble 1). All patients had previously received radiotherapy at some point during their treatment. Entry into the surgical treatment arm required only that the tumor could be resected to microscopic residual disease (without resection of the internal carotid artery), but resection offered little or no opportunity for cure as determined by the Multidisciplinary Head and Neck Oncology Treatment Planning Committee at The University of Texas M. D. Anderson Cancer Center. There were 10 males and 5 females, with a mean patient age of 54.3 years. Tumor *p53* genotype was analyzed (by direct sequencing) for each patient, although a mutant genotype was not a prerequisite for study entry. Patients were required to practice contraception while in the study, and women of child-bearing age had to have negative pregnancy tests. A detailed description of the 15 subjects can be found in Table 1. The study was reviewed and approved by the Institutional Surveillance Committee of The University of Texas M. D. Anderson Cancer Center, the NIH Recombinant DNA Advisory Committee, and the Food and Drug Administration. Informed consent was obtained from all patients before study entry, with emphasis placed upon the investigational nature of the study and the absence of therapeutic intent.

Recombinant Adenovirus. The recombinant adenovirus Ad-*p53* was used to directly introduce the wild-type *p53* gene into all subjects. Preparation of the recombinant adenovirus was described previously (18). Ad-*p53*, also designated as INGN201, is a replication-defective adenovirus serotype 5 vector with a cytomegalovirus-promoted *p53* cDNA insert replacing the E1 region of the vector. Ad-*p53* is a BL-2 agent and was handled with the appropriate level of biological containment. Ad-*p53* was produced by Magenta, Inc. (now MA Biosciences, Rockville, MD) and Introgen Therapeutics (Houston, TX) and stored at −80°C at concentrations of 2×10^{10} to 3×10^{10} pfu/ml in PBS supplemented with 10% glycerol. Ad-*p53* was thawed and diluted in PBS at 4°C within 2 h of use.

Administration of Ad-*p53*. All Ad-*p53* was administered on an inpatient basis under strict aseptic conditions. Ad-*p53* was delivered to sites of disease recurrence only. There were three Ad-*p53* intervention approaches/patient: (*a*) preoperative; (*b*) intraoperative; and (*c*) postoperative.

Ad-*p53* was given in escalating doses to determine a maximum tolerated dose for this treatment strategy. The Ad-*p53* dose did not vary throughout each patient's treatment (Table 2). Doses started at 1×10^6 pfu and were increased in log increments until 1×10^9 pfu was reached and then increased in one-half log increments until 1×10^{11} pfu was reached.

The preoperative Ad-*p53* administration consisted of direct tumor injections given three times weekly for 2 consecutive weeks (six treatments overall). The preoperative injection volumes were based on the estimated volume of the injected mass and the number of injection sites. Ad-*p53* was administered using 27-gauge needles and 3–10-ml syringes, depending on the volume injected. Ad-*p53* was injected directly into tumors by inserting the needle to the tumor depth and injecting upon withdrawal. Ad-*p53* was diluted in a volume of PBS concordant with the number of tumor injections to be performed. Generally, we injected about 0.5 ml of vector solution at 1-cm (surface area) tumor increments. Thus, a very large tumor required the appropriate amount of vector to be diluted in a larger volume of PBS. Tumor maps were generated depicting the injection sites so that these areas could be reinjected. A typical tumor map for a recurrent oral tongue lesion is shown in Fig. 1.

Seventy-two h after the last Ad-*p53* intratumoral injection, patients had their surgery. At the time of surgery, after total gross tumor removal and just before closure, another dose of Ad-*p53* (diluted to 10 ml in PBS) was delivered by injection to the surgical sites where microscopic residual disease was presumed to be present, including mucosal margins of the resected neoplasms (Fig. 2). A small amount of this dose was saved and administered liberally (a vector wash) to the tumor bed via a syringe and left in contact for 60 min before wound closure.

Seventy-two h after surgery, the patients received the final Ad-*p53* administration (again diluted to 10 ml in PBS) via retrograde instillation through wound catheters that had been placed intraoperatively in the areas of presumed microscopic residual disease. Clamps were used to prevent efflux of the Ad-*p53* for 1 h. The drains were removed 24–48 h after the postoperative instillation.

Statistical Analysis of Patient Outcome. Kaplan-Meier disease-specific survival and disease-free intervals were analyzed for all 15 patients entered into the surgical arm of the study. The time of study entry was the day of the first preoperative Ad-*p53* administration. All patients were macroscopically free of disease after surgical resection.

Patient Monitoring. Because the treatment of patients with Ad-*p53* was within the context of a Phase I clinical trial, diligent patient monitoring for the detection of untoward and toxic effects was obligatory. Surgical complications as well as potential Ad-*p53*-related toxic effects were recorded. Vital signs were recorded, performance status was evaluated, and chest X-rays and hematology and blood chemistry testing were performed daily. Patients were closely monitored for 2 h after each Ad-*p53* administration.

Detection of Wild-Type p53 and $p21^{Waf1}$ Gene Product Expression and Apoptosis. Biopsy samples taken from the tumor margins of a representative nonsurgical patient were analyzed 48 h after Ad-*p53* delivery (10^6 pfu) to the tumor. This immunohistochemical analysis examined the expression of the wild-type p53 gene product and the gene product of the downstream p53-transactivated gene $p21^{Waf1}$ (19) via an avidin-biotin-peroxidase complex method (20). The DO-1 anti-p53 mouse monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and the anti-$p21^{Waf1}$ mouse monoclonal antibody (Oncogene, Uniondale, NY) were used for all p53 and $p21^{Waf1}$ immunohistochemical studies, respectively. Standard H&E staining as well as TdT end-labeling to detect apoptotic cells were performed on similarly prepared tumor margin biopsy samples 48 h after Ad-*p53* delivery to the tumor. TdT end-labeling was performed with the ApoTag Plus kit (Oncor, Gaithersburg, MD) according to the manufacturer's instructions. All of the these studies were matched with biopsy samples taken from adjacent uninjected grossly normal tissues of the same patient 48 h after Ad-*p53* delivery to the tumor.

RESULTS

Patient Outcome. The Kaplan-Meier disease-specific survival curve for the patients enrolled in the surgical arm of the

Table 2 Ad-*p53* and surgical treatment and related complications of surgical treatment arm study participants

Patient no.	Ad-*p53* dose per treatment 1×10^x pfu	Adenovirus complications	Surgical treatment of recurrence	Surgical complications	Current disease status
1	6	Headache with first preoperative injection	Left maxillectomy and mandibulectomy, bilateral NDs,[a] total laryngectomy, partial pharyngectomy, latisimus free flap and bilateral pectoralis flap reconstruction	Postoperative fever with positive blood cultures for *Staphylococcus*	DOD
2	6	Erythema at preoperative injection site	Total glossectomy, total pharyngectomy, bilateral NDs, resection anterior neck skin, right pectoralis flap reconstruction	Intraoperative bradycardia and atrial flutter, electrolyte imbalance	DOD
3	7	None	Left extended RND, left pectoralis flap reconstruction	None	NED
4	7	Pain with preoperative injections	Total laryngectomy, total glossectomy, right MRND, marginal mandibulectomy, left verticle rectus myocutaneous flap reconstruction	None	DOD
5	8	None	Total pharyngectomy, cervical esophagectomy, completion thyroidectomy, bilateral MRND, mediastinal nodal dissection, free jejunum reconstruction	Anemia, electrolyte imbalance, confusion, fever, mild respiratory insufficiency, acute renal insufficiency	NED
6	8	None	Total laryngectomy, right MRND, left RND, partial pharyngectomy, subtotal thyroidectomy, total glossectomy, free rectus flap and pectoralis flap reconstruction	Anemia, electrolyte imbalance, acute renal insufficiency	DOD
7	9	Tenderness at preoperative injection site	Left extended RND, pectoralis flap reconstruction	Anemia, electrolyte imbalance, aspiration pneumonia	DOC
8	9.5	Fever during early preoperative injections, pain with preoperative injections	Right ND	None	DOD
9	10.5	Sore throat, increased dysphagia, sinus congestion, and headache during preoperative injections	Total pharyngectomy, total thyroidectomy, bilateral MRND, free jejunum reconstruction	Anemia, electrolyte imbalance, ascites, hypothyroidism, pneumatosis intestinalis	DOD
10	10.5	Fever, sore throat, headache, and increased odynaphagia and dysphagia during preoperative injections	Partial glossectomy, hemimandibulectomy, left MRND, free fibula osseo-cutaneous flap reconstruction	Anemia, electrolyte imbalance, hypertension, pneumonia, delayed cervical wound healing	DOD
11	10.5	Fever during preoperative injections, and pain associated with injections	Left partial pharyngectomy, left partial mandibulectomy, left RND, right verticle rectus free flap reconstruction	Anemia, electrolyte imbalance, pleural effusion, pneumonia and respiratory failure, hypertension, cellulitis left neck	DOC
12	11	Pain after preoperative injections, throat swelling after first preoperative injection	Total glossectomy, total laryngectomy, partial pharyngectomy, partial mandibulectomy, bilateral ND, free transverse rectus abdominis flap reconstruction	Anemia, electrolyte imbalance and hypovolemia, fever	DOD

Phase I Ad-*p53* clinical trial is shown in Fig. 3. Median survival was 12.4 months. Currently, four patients are alive with no evidence of disease (at 29.1, 23.8, 11.5, and 12.7 months). One patient is alive with disease (at 13.1 months), and eight patients have died of disease. Two patients died of unrelated causes (at 13.4 and 4.8 months). The current disease status of each study participant is shown in Table 2.

The median disease-free interval was 3.9 months for the nine patients enrolled in the surgical treatment arm whose disease recurred. The four patients without evidence of disease (disease free at 29.1, 23.8, 11.5, and 12.7 months) and the two patients who were without evidence of disease at the time of death (at 13.4 and 4.8 months) were not included in this calculation.

Table 2 Continued

Patient no.	Ad-*p53* dose per treatment 1×10^x pfu	Adenovirus complications	Surgical treatment of recurrence	Surgical complications	Current disease status
13	11	Fever after first two preoperative injections, erythema and induration at preoperative injection sites, headaches postinjection	Total glossectomy, total laryngectomy, partial mandibulectomy, bilateral ND, free rectus flap reconstruction	Anemia, electrolyte imbalance, breakdown of flap reconstruction	NED
14	11	Fever and headache following preoperative injections	Total laryngopharyngectomy, left MRND, free jejunum reconstruction	Electrolyte imbalance, respiratory failure, hypothyroidism and hypoparathyroidism	NED
15	11	Fever after first preoperative injection, erythema and induration at preoperative injection site	Resection left buccal mucosa, partial maxillectomy, partial mandibulectomy, infratemporal fossa resection, left MRND, free flap reconstruction	Electrolyte imbalance, flap hematoma, agitation and confusion	AWD

[a] ND, neck dissection; AWD, alive with disease; DOC, died of other causes; DOD, died of disease; NED, no evidence of disease; RND, radical neck dissection; MRND, modified radical neck dissection.

Surgical Complications. Despite the extensive prior treatments and often tremendous tumor burdens, surgical complications in the context of Ad-*p53* administration were relatively minor among the 15 study subjects, considering the extent of resection in most cases. There was one instance of delayed wound healing (patient 10) and one instance of flap breakdown (patient 13) requiring operative revision. There were no fistulas or wound infections. A detailed list of the surgical procedures for each patient, along with related complications, is provided in Table 2. The most common complications were electrolyte imbalance (usually consisting of transient hypokalemia or hypomagnesemia due to prolonged anesthesia; 11 of 15 patients), anemia (8 of 15 patients), pneumonia (3 of 15 patients), acute renal insufficiency (2 of 15 patients), and transient hypothyroidism (2 of 15 patients). All complications resolved with appropriate fluid or pharmacological intervention or both.

Ad-*p53*-related Complications. The dose of Ad-*p53* that was administered to each patient is indicated in Table 2, along with a detailed list of treatment-related sequelae. All Ad-p*53*-related complications occurred during the preoperative administrations and were mild. All patients were able to tolerate the full course of Ad-*p53* interventions (preoperative, intraoperative, and postoperative). As can be seen in Table 2, Ad-*p53*-related complications were more frequent at the higher viral doses ($\geq 1 \times 10^9$ pfu). Fever after Ad-*p53* administration was the most common finding, occurring in six patients. Fever was not observed in patients who received less than 1×10^9 pfu/dose and was only transiently observed after the first injection or the first and second injections during preoperative administration. Fevers ranged from 38.1°C in patient 11 to 39.4°C in patient 10. Pain at the site of injection was also a frequent finding, occurring in five patients. This sequela was believed to be related to the cold temperature of the injected Ad-*p53* solution. In patients 9, 10, 12, and 13, mild, transient, flu-like symptoms were observed early in their preoperative Ad-*p53* administration courses.

Gene Product Expression and Induction of Apoptosis. Dark green positive immunohistochemical stainings for the wild-type p53 gene product (Fig. 4*D*) and the $p21^{Waf1}$ gene product (Fig. 4*F*) were demonstrated at the tumor margins of an Ad-*p53*-treated tumor from a representative nonsurgical patient. Matched samples from adjacent untreated normal tissue (Fig. 4, *C* and *E*) stained negatively. Only mild suprabasal detection of endogenous p53 was detected in the untreated tissue (Fig. 4*C*). It should be noted that the wild-type p53 gene product was detected despite the presence of a rigorous immune infiltrate (and systemic anti-adenovirus antibody titer; data not shown) seen on a posttreatment H&E-stained section from the tumor margin (Fig. 4*B*). Fig. 3*H* shows the brown-stained apoptotic tumor cells in the submucosa (by TdT end-labeling assay) present in a biopsy sample of the tumor margin after Ad-*p53* injection, relative to the matched biopsy of adjacent untreated normal tissue (Fig. 4*G*).

DISCUSSION

Because of its propensity for locoregional recurrence and poor survival, SCCHN remains a devastating disease, despite treatment advances (1, 2, 5). A major factor leading to locoregional recurrence of SCCHN is microscopic residual disease after definitive surgery, radiotherapy, chemotherapy, or any combination of the three. Even histologically "normal" tissue at the margins of tumor resection can harbor molecular characteristics that portend disease recurrence (3, 4).

The situation of patients with locoregionally advanced SCCHN who have unsuccessfully undergone other therapies, including radiotherapy, is particularly problematic. Additional chemotherapy does not seem to offer a significant survival advantage to these patients (21), and they have few viable treatment options, even when tumors are surgically resectable. Thus, we selected this population of patients for our Phase I clinical trial of Ad-*p53* gene therapy. The known role of *p53* as a tumor suppressor gene and an inducer of cell cycle arrest and apoptosis in mammalian cells (6, 13–16), as well as our encouraging preclinical *in vitro* and *in vivo* animal findings with Ad-*p53* in SCCHN (7–9), made this an attractive treatment strategy.

As indicated earlier, the Phase I study of patients with

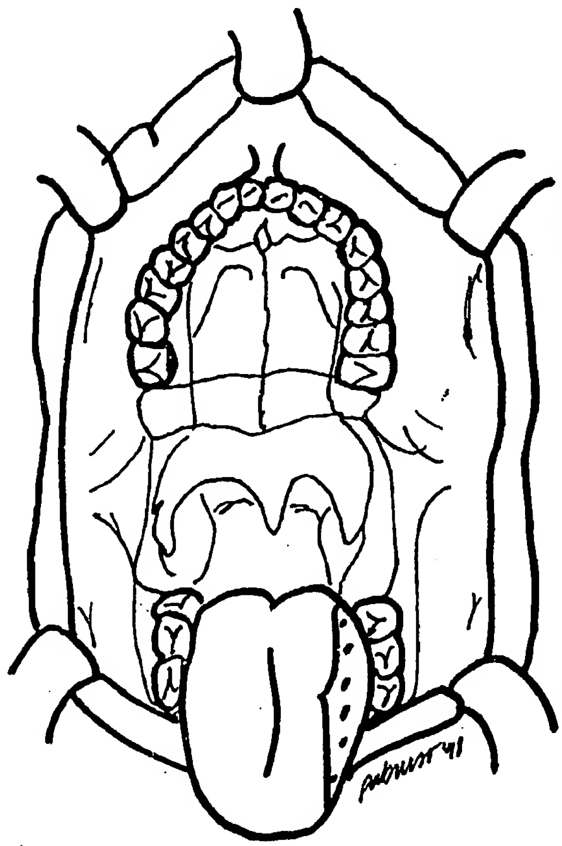

Fig. 1 Example of a typical tumor map for a left tongue carcinoma. Note the incremental markings along left tongue lesion indicating sites where Ad-*p53* is injected.

Fig. 2 Intraoperative delivery of Ad-*p53* to the tumor bed. Ad-*p53* is being injected into the tumor margins before a vector wash of the tumor bed.

advanced locoregionally recurrent SCCHN revealed that Ad-*p53* gene transfer is safe and well tolerated (17). Furthermore, in the current analysis, apoptosis and expression of the wild-type p53 and p21^{Waf1} (a downstream p53-transactivated gene) gene products were demonstrated in tumor margin biopsy samples taken from a representative nonsurgical patient after Ad-*p53* delivery. The findings with regard to median survival in the surgical arm of the study (Ad-*p53* delivered as an adjuvant to surgical therapy) prompted the current report, although our sample size was small, and thus the results should not be overinterpreted. The median survival for these patients (12.4 months) was about 60% longer than that found in chemotherapy trials for similar patients (21). Furthermore, the median disease-free interval of 3.9 months among those patients whose disease recurred suggests that this trial was not preselecting a favorable patient population. The observations made with regard to potential antitumor activity among patients with resectable tumors is encouraging as we proceed with the international Phase II evaluation of Ad-*p53* gene transfer in patients with SCCHN. Recurrence rates and mortality are higher in patients with molecular evidence of residual disease (as determined by PCR-based assay of *p53* mutation) at tumor margins (1, 2). Thus, the use of Ad-*p53* as an adjuvant modality in surgical wound beds may lower those rates.

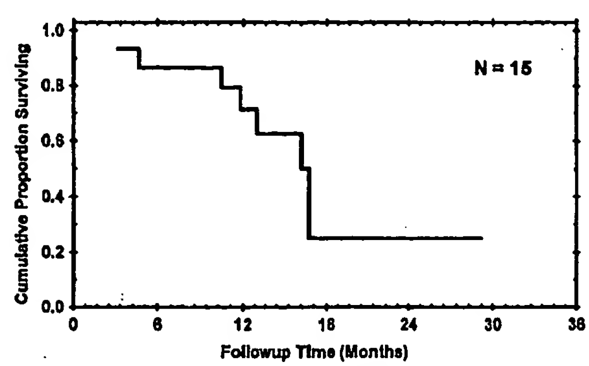

Fig. 3 Kaplan-Meier survival curve for patients in the surgical treatment arm.

There are several implications of our findings. Given the low toxicity of Ad-*p53*, this agent may be applied as an adjuvant therapy after primary definitive treatment of advanced lesions (or early lesions), as indicated above. Furthermore, Ad-*p53* gene transfer may be efficacious in dysplastic lesions because *p53* mutations have been found in head and neck premalignancies

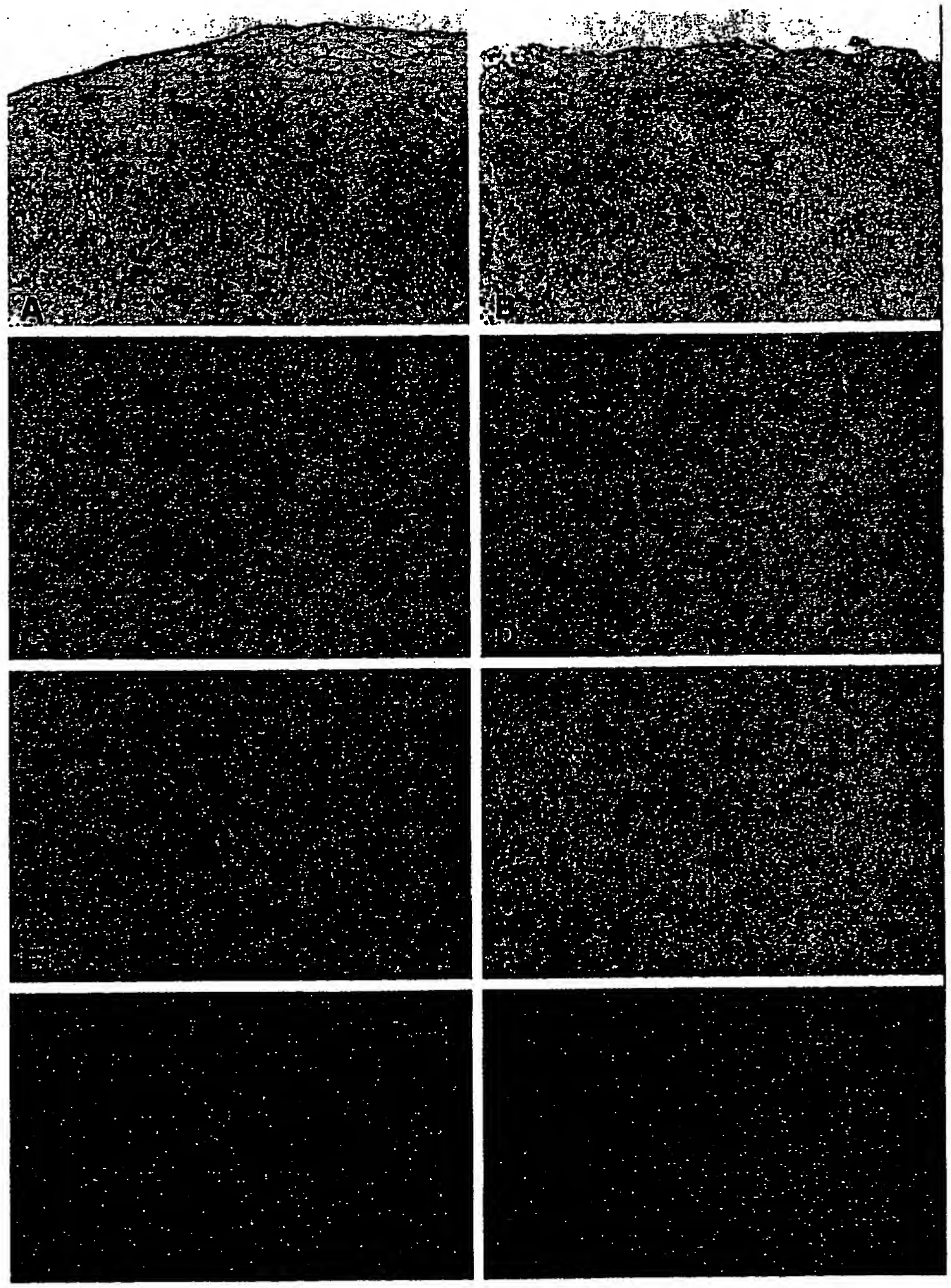

Fig. 4 Immunohistochemical, H&E, and TdT end-labeling analyses of biopsies taken from the tumor margins of a representative nonsurgical patient 48 h after Ad-*p53* delivery to the tumor. In tumor margin biopsy samples, immunohistochemical staining for expression of the wild-type p53 gene product (*D*) and p21^{Waf1} gene product (*F*) is shown 48 h after Ad-*p53* delivery to the tumor. *C* (immunostained for expression of the wild-type p53 gene product) and *E* (immunostained for expression of the p21^{Waf1} gene product) show matched biopsy samples taken from untreated normal tissues 48 h after Ad-*p53* delivery to the tumor. In tumor margin biopsy samples, H&E staining (*B*) and TdT end-labeling (*H*) are shown 48 h after Ad-*p53* delivery to the tumor. *A* (stained with H&E) and *G* (end-labeled with TdT) show matched biopsy samples taken from untreated normal tissues 48 h after Ad-*p53* delivery to the tumor.

(22). Finally, Ad-*p53* gene therapy may be applied in combination with radiotherapy or chemotherapy because enhanced antitumor activity has been seen in such combination treatment models in preclinical studies (23, 24).

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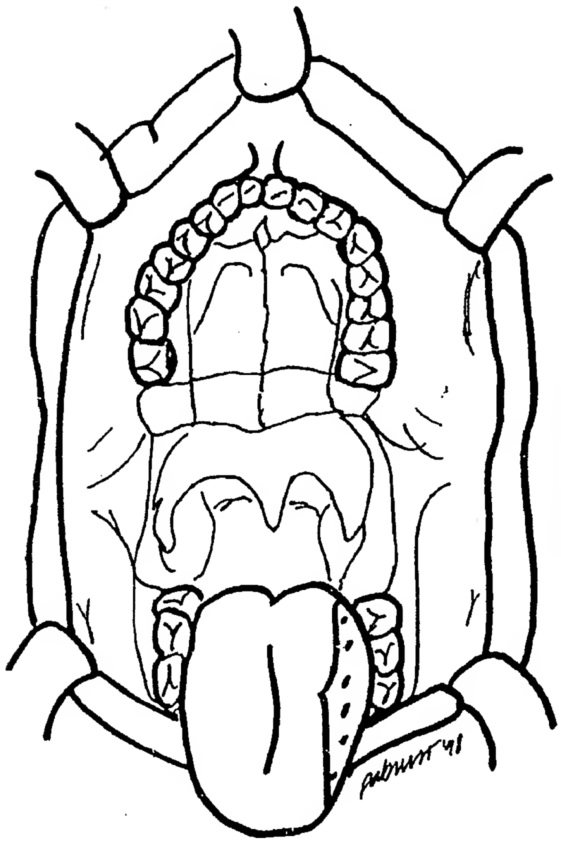

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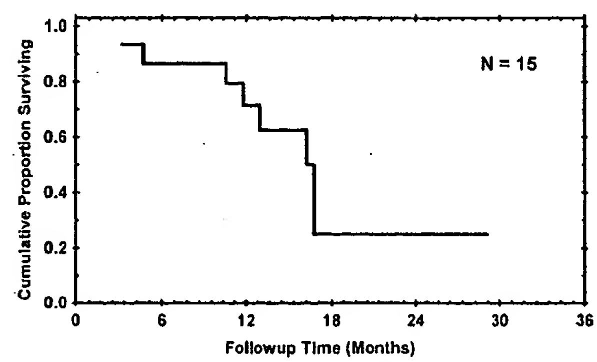

Fig. 3 Kaplan-Meier survival curve for patients in the surgical treatment arm.

There are several implications of our findings. Given the low toxicity of Ad-*p53*, this agent may be applied as an adjuvant therapy after primary definitive treatment of advanced lesions (or early lesions), as indicated above. Furthermore, Ad-*p53* gene transfer may be efficacious in dysplastic lesions because *p53* mutations have been found in head and neck premalignancies

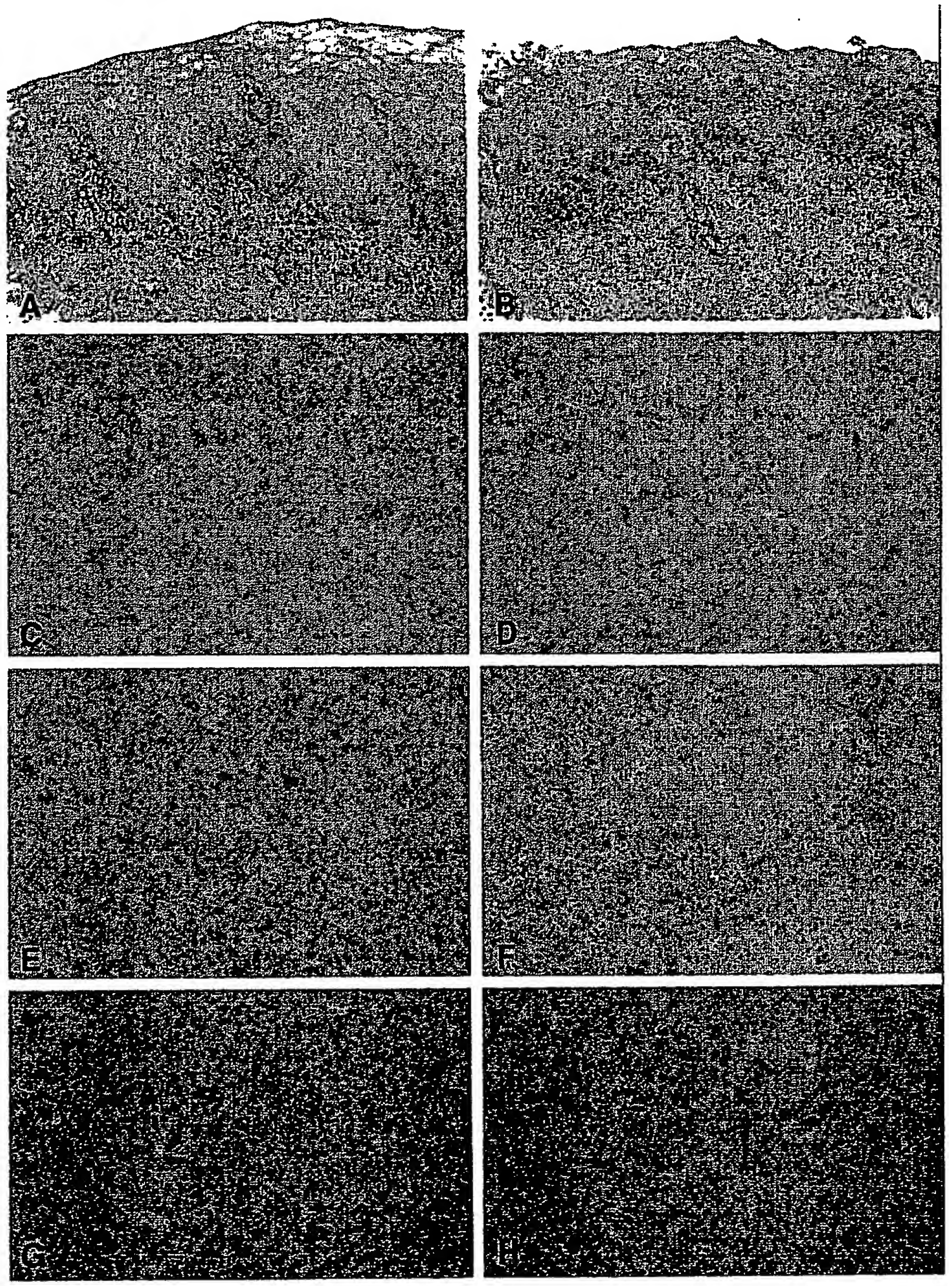

Fig. 4 Immunohistochemical, H&E, and TdT end-labeling analyses of biopsies taken from the tumor margins of a representative nonsurgical patient 48 h after Ad-*p53* delivery to the tumor. In tumor margin biopsy samples, immunohistochemical staining for expression of the wild-type p53 gene product (*D*) and p21^{Waf1} gene product (*F*) is shown 48 h after Ad-*p53* delivery to the tumor. *C* (immunostained for expression of the wild-type p53 gene product) and *E* (immunostained for expression of the p21^{Waf1} gene product) show matched biopsy samples taken from untreated normal tissues 48 h after Ad-*p53* delivery to the tumor. In tumor margin biopsy samples, H&E staining (*B*) and TdT end-labeling (*H*) are shown 48 h after Ad-*p53* delivery to the tumor. *A* (stained with H&E) and *G* (end-labeled with TdT) show matched biopsy samples taken from untreated normal tissues 48 h after Ad-*p53* delivery to the tumor.

(22). Finally, Ad-*p53* gene therapy may be applied in combination with radiotherapy or chemotherapy because enhanced antitumor activity has been seen in such combination treatment models in preclinical studies (23, 24).

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